OBSERVATIONS ON THE LIFE HISTORY OF
THE MARBLED SALAMANDER, *AMBYSTOMA OPACUM* GRAVENHORST

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(Figs. 83–93 incl.)

INTRODUCTION

The Marbled Salamander, *Ambystoma opacum,* is one of the commonest species of *Ambystoma* in the eastern United States. It differs from all other species of the genus in its habit of usually laying its eggs on land. This fact, first discovered by the Reverend Charles Mann in 1855, has excited the attention of herpetologists for many years. Many observations have been reported on the life history of this form, those of Dunn (1917), Brimley (1920), Bishop (1924), and Lantz (1930 and 1930a) being especially noteworthy. In 1929, while collecting in the vicinity of Washington, D. C., we discovered that great numbers of brooding females could be obtained under leaf mold on dry pond bottoms or on pond banks which later would be flooded. The fact that we could obtain eggs in large numbers induced us to repeat the work of previous observers and to examine certain additional features of the life history. We have already published a note (Noble and Brady, 1930) on certain phases of this work and in this paper we intend to put on record a summary of our observations extending over a period from October 1929 to November 1931.

In the course of the work we received assistance from several sources. We are especially indebted to Mr. Sam Yeaton, Mr. G. P. Engelhardt, and Mr. J. A. Weber for the opportunity of publishing their field notes, which are recorded below. Miss Margaret Harland has given us technical aid in the preparation of the material and we are indebted to her for the microphotography.

Fig 83.   Marbled salamander brooding her eggs.   The cover of moss has been removed but the female has not changed her position.   The female usually lies adjacent to the egg mass while brooding and rarely does the tail cover the egg mass as shown here.   Photographed at Miller's Place, Long Island, by S. C. Yeaton.

BREEDING TIME AND PLACE

The eggs of *A. opacum* have been collected during all the fall months.   The Reverend Mann (1855) found eggs at Gloucester, Virginia, in November and December.   McAtee (1907) gives September and October as the months during which this species is found with eggs in Monroe County, Indiana.   Brimley (1920) records eggs in October and November in the region near Raleigh, North Carolina.   Dunn (1917) found fresh eggs at Mt. Vernon, Virginia, September 21, 1916.   Deckert (1916) records eggs with developed embryos from Silver Lake, Westchester Co., New York, September 25, 1913.   Bishop (1924) took two sets of eggs at Raleigh, North Carolina, on October 26, 1923.   Eggs which we collected from October 3 to 6, 1929, in the Washington region had already undergone considerable development.   On Long Island during 1931, egg-laying had occurred in damp locations by September 18.   By September 20, most females discovered were with their eggs (Fig. 83).   On the other hand, Mr. G. P. Engelhardt writes us that he has had a female under observation in the field at Hartsdale, New York, which laid eggs between October 2 and 4, 1931.   It may be concluded that both in the New York and in the Washington areas egg-laying may occur in the field as early as September 20; in Westchester County it may occur as late as the first week in October.   Females, which we brought from Long Island into the laboratory, laid eggs as late as September 28, 1931.   Lantz's

specimens which were collected in the Washington area laid eggs in England on October 10. Mr. J. A. Weber has reported to us his discovery of six clutches of eggs with attendant females at Greensburg, Louisiana, October 25, 1930. From the form of the eggs he assumed that they had been laid recently.

Until the time we began our work with *A. opacum* it seemed that most eggs of the species had been discovered under logs or similar coverings by the edge of ponds, or more often under similar materials lying in hollows which later would be filled with rain water. Consequently we were interested to discover that the species lays its eggs far more frequently under dead leaves or grass in regions which later will be inundated. Several of these breeding sites may be considered in detail because the species will doubtless be found abundant elsewhere among similar surroundings.

In the Washington area the two localities studied during 1929 differed considerably in physiography. The breeding ground at Oakwood, Virginia, was in the dry bed of a temporary pond on one side of a railroad embankment. The region we studied covers about one-quarter of an acre and is of relatively recent origin. It is filled to varying degrees with rain water from December to June. The Rock Creek Park territory investigated was formed by the dry banks of a permanent pond, exposed by the autumnal drought. The terrain of both sites consisted of a layer of matted grass and leaf mold, covering a rather plastic clay. Much effort was spent at Oakwood in turning over logs. However, since but two nests were secured this way, in a spot where the form was known to be abundant, it was decided to investigate the mat of leaf mold which covered the lower areas of the pond floor. When this was rolled back a great ramification of tunnels was exposed. The mat of leaf mold had formed the roof of these tunnels. At various points along these runways, such as depressions or blind wings off the main corridors, the egg-clutches with attendant females were observed. The depressions or burrows containing the clutches were never very deep and the eggs were visible the moment the leaf-mold roofing was removed. Clutches were found occasionally around the entrances of holes, presumably made by crayfish, which opened up into the tunnels, and a few eggs were found within the holes. There was no evidence that the female had pushed or carried these eggs into these deeper tunnels, since the greater mass of every clutch was always found in the surface depression under the leaf mold. It was apparent, however, that the female had something to do with the shape of the immediate depression in which the eggs were deposited. In the majority of cases the area surrounding the eggs had the appearance of having been smoothed out and the edges of the depression quite often shelved out over the egg mass. Lantz says his female "slightly excavated the ground under a creeping plant" in the terrarium. It cannot be said with certainty that the depressions and tunnels found in the clay subsoil were originally made by the females. The soil was relatively resistant and could not be easily scooped out by a salamander. It seems more probable that most of the tunnels originally were made by some other agency but apparently they owed their smoothed-out and well-worn aspect to the activities of the brooding females. Unfortunately the ability of the females to excavate their nests in the hard clay could not be determined in the laboratory since our specimens had all laid and in the absence of any greater impulse were

content merely to tunnel in the moss and overlying debris of the terrarium. In some instances the nests were made in very faint depressions unconnected with any of the surface tunnels. Three such nests found at Oakwood had broad, well-smoothed edges and it is quite possible that they were excavated by the females. Several males and one spent female were found around the edges of the nesting area at Oakwood. None of these had excavated chambers such as those in which the eggs were found.

The selection of the nest site itself apparently involves the presence of a certain amount of moisture in the substratum and a cover of dead leaves or other material. At Oakwood the nests were not distributed evenly over the entire floor of the pond but were found clustered together in spots. These places usually were around the bases of alders, or along depressions made by drainage from the forest floor above the pond. Such places were more moist and had more leaf-mold covering than had other portions of the pond bottom. Clusters of from twelve to twenty nests would thus be found in certain favorable spots. In the Oakwood section one area 300 cm. long and 60 cm. wide, between the bases of two alders, yielded eighteen nests. Another area 150 cm. square yielded twenty nests. In some cases it was impossible to separate the clutches, two or three masses being confluent along the burrow floor. In the Rock Creek station a small area 109 cm. by 62.5 cm. contained seven nests, while a carefully examined area of 18 meters square on either side yielded but three nests. Here it may have been a question of cover. The leaf mold was thickest in the area in which the seven nests occurred. Further, this small area was situated on the steep slope of the pond side, three of the nests being found 109 cm. from the edge while the others were strung out between this point and the edge. There were no nests on the flat surface of the forest floor above. This seems to indicate that low depressions are not invariably selected, but that the character of the cover and degree of moisture are important factors in the selection of the breeding site. Invariably the nests were in an area later to be submerged by the early winter level of the pond.

Our method of hunting for the nests was as follows: On moving over the surface of the ground on hands and knees we would feel for a slight "give." This would indicate the presence of a burrow mouth or depression under the leaves. As such situations usually contained nests, our hunting on October 4 consisted of feeling for such spots. The method of feeling for nests was finally abandoned because we found that pushing in the nest roof tended to disturb the brooding female. We first developed a much better method while studying one of the ponds at Coram, Long Island, during 1930. Here the water vegetation had become matted down and dried during the summer drought. Standing in the deeper parts of the depression, we cut a strip of this matting three or four feet wide and rolled it back towards the higher ground. A great many nests were found scooped in shallow depressions in the dirt floor of the pond, and were fully exposed as the matting was rolled back.

Mr. Sam Yeaton has examined other localities on Long Island where other methods of hunting had to be employed. His field notes are also of interest in indicating the importance of humidity in regulating the nesting site selection. At Lake Grove, on September 18, 1931, he found:

"The bottom of the pond was sandy, very dry and barely covered with humus. Thirty adults were found, usually in small groups in the side of tunnels formed by mice or possibly by the salamanders themselves. Most of the salamanders were about three inches below the surface of the sand. No eggs were found here, although we found over twenty gravid females. Two specimens were discovered about six inches from the ground in the low branches of shrubs. A female with a nest of eggs was found the same night at Coram near a small back-water between Coram and Middle Island.

"On September 19, 1931, eighty-three adult *opacum* were caught at Jones Pond, Miller's Place. Only a dozen of the females were brooding eggs but the remainder were swollen with eggs. The brooding females had scooped out shallow depressions beneath the sheets of matted grass where the ground was still moist. The bottom of this pond was very flat and the nests were scattered all over the pond floor and not concentrated at certain levels as is the rule in the case of nests in dry ponds having steeper banks. In many cases the males and females were found together. Some of the depressions in which the eggs lay were natural irregularities of the pond floor but others apparently had been dug by the female. Some of the females without eggs had dug shallow depressions and apparently were preparing to lay eggs in them. On April 4, 1931, Mr. Jay A. Weber and I dredged this area without finding any *opacum* larvae. This would indicate that the adults had not laid here the previous fall in spite of their present abundance.

"On September 21, 1931, near Melville, Long Island, thirty-three adults were collected, mostly brooding females. Many females with eggs were on a moist bank in depressions made by the hoofs of cows. On September 18, we had visited the same locality and found no eggs. Males were found wandering in damp places near the border of the ponds while the females were restricted to suitable breeding grounds."

These data indicate that the female *opacum* may change her breeding site from season to season according to the degree of humidity present in any one locality. The largest number of brooding females collected by Mr. Yeaton during 1931 was obtained under sheets of grass or other vegetation in situations similar to those at Coram. In 1930 at this locality we also found females brooding their eggs under sheets of sphagnum. In 1931 Mr. Yeaton collected forty-two adults in this habitat. The females in one area showed a decided preference for sphagnum covering the roots of fallen trees. The nest level averaged 30 cm. above the dry swamp bottom but because of the pitch of the surrounding banks these roots would be inundated by the first heavy rains.

Mr. Yeaton made an especial effort to collect migrating *opacum* for it was obvious that some specimens could not have been in their breeding sites all summer. On September 19, 1931, near Miller's Place, Long Island, he found one gravid female beneath a stump on a gravelly hill-side about 180 m. from water and on the other side a road separating it from any marshy places. Another specimen was discovered on the same day about one and one-half miles from the nearest water. There was a dry pond about 60 m. from this place but it could not have held more than 30 cm. of water or have been more than 450 cm. square. No salamanders were found in this dry bottom and it is doubtful if the captured female would have laid her eggs there.

In 1932, after the present manuscript had been submitted for publication, Mr. Yeaton made another visit to Miller's Place with a view to observing a more extensive migration of *A. opacum* to the breeding areas. In this he was unsuccessful, but on September 9 he collected at Miller's Place no less than eighty-five males without finding a single female. There were no eggs or females in this area which he knew from previous experience would be later occupied by brooding females. Two days later the first females were captured in this area. It is therefore clear that the males of *A. opacum*, like the males of *A. maculatum*, precede the females to the breeding sites. This precedence of the males over the females appears to be the rule of many migratory salamanders as well as of frogs, of birds and of some mammals.

These data, although scanty, indicate that both sexes of *opacum* migrate to suitable breeding grounds about the middle of September, the males arriving before the females. The eggs are laid on land in moist situations. If the locality is very dry, egg laying does not occur. Possibly the adults move on toward more suitable situations. We have seen no evidence of a mass migration such as occurs in *Ambystoma maculatum*. Since courtship, as we shall show below, is not performed by great numbers of individuals at one time such mass movements would not be expected.

The courtship of *A. maculatum* occurs in the water. This species does not appear to be better fitted structurally for aquatic life than does *A. opacum*. We were interested in testing the reactions of the latter species to submergence, for it seemed possible that these reactions might throw some light on the breeding behavior of the species. We carried out our first experiments in the field.

At the Rock Creek station a brooding female was placed in water several centimeters deep and was observed to swim clumsily to the surface, endeavoring to keep her head out. She swam in various directions but upon reaching an object which provided a foothold she immediately climbed out of the water, instead of seeking to escape under the submerged leaves and debris as *Ambystoma maculatum* would have done. In the laboratory, the behavior of *A. opacum*, both male and female, when placed in water 10 cm. in depth, were in marked contrast to the behavior of *A. maculatum* and *A. tigrinum*, under the same circumstances. *Tigrinum* was found to swim readily about the tank, eagerly accepting food offered to it under water. *Maculatum* was equally at home in the water, although it was not capable of the same ease in swimming. *Opacum*, however, under these circumstances, showed signs of distress, swimming violently and clumsily about the tank, endeavoring to escape by climbing movements of the limbs. Three pairs of *opacum* were placed in a tank with a water depth of 2 cm. and provided with a stone shelter, the top of which was near the surface of the water. Under these conditions the animals were always able to keep their heads above the water and walked leisurely about seeking an escape, when they were not resting on the stone work. Next day the water depth was increased 3 cm. The animals immediately made a more violent effort to escape, striving to keep their heads above water. When the depth was increased to 15 cm. two of the animals drowned; the remaining four, by dint of stretching up from the rock work, were able to continue atmospheric respiration. When the depth was reduced to 2 cm. the specimens moved about in a leisurely manner. Specimens

kept for a month in water having a depth of 2 to 3.5 cm. never became completely acclimated to that element, refused food and consistently endeavored to escape. From this it would seem that *A. opacum* is certainly not at home in the water and will drown if denied the opportunity to indulge in atmospheric
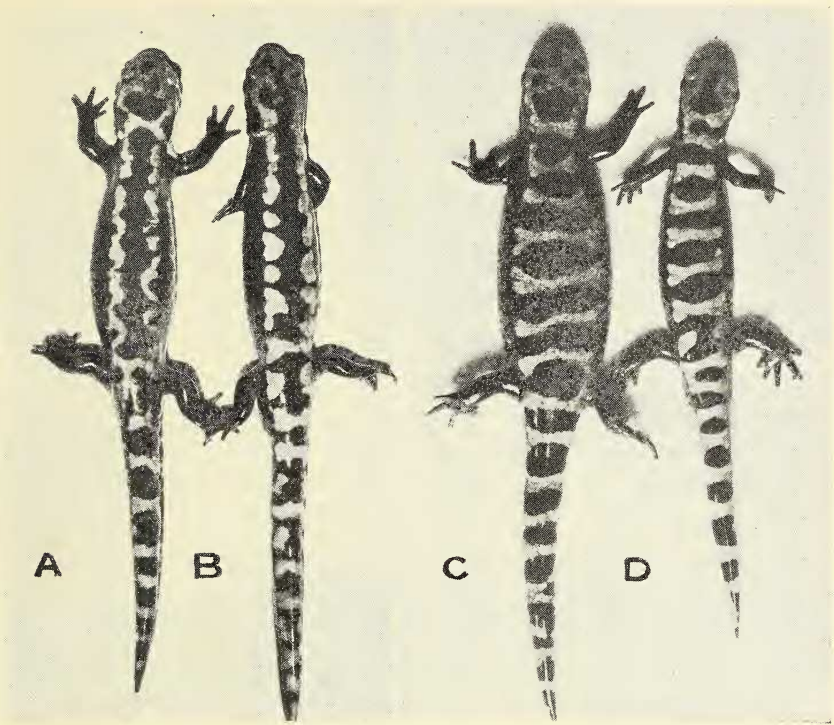

Fig. 84. Two pairs of *Ambystoma opacum* collected on Long Island showing extremes of variation in color pattern. Females (A and C) may be striped or cross-barred. Their color pattern does not distinguish them from the males (B and D).

respiration while in that element. It is certain that in nature the species never deposits its eggs in the water and that the female must desert her nest as soon as the pond basin begins to fill with water.

SEXUAL DIFFERENCES IN COLORATION

Ambystoma opacum is unique among the species of the genus in exhibiting a consistent difference in color between the sexes during the breeding season. The white color pattern along the back is distinctly whiter in courting males than in females. Nevertheless, as indicated below, we have failed to find that this color difference plays any role in sex recognition or courtship.
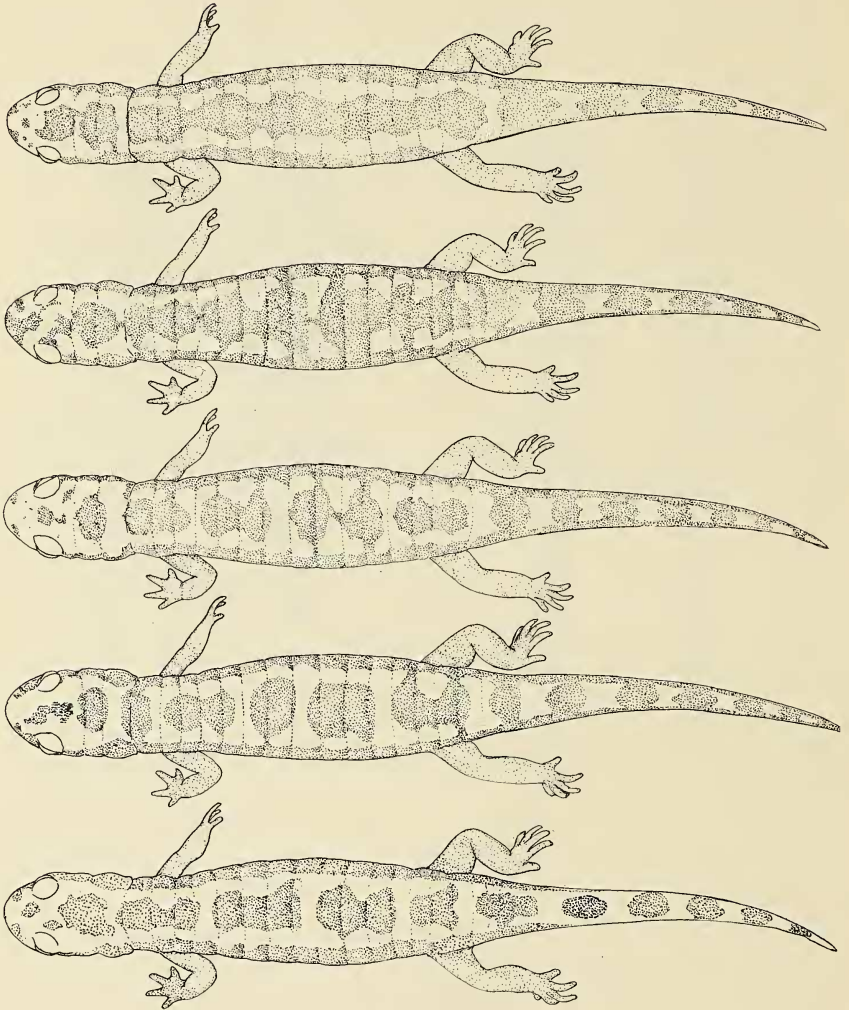
Fig. 85.   A series of brooding *Ambystoma opacum* collected in the vicinity of Washington, D. C., illustrating the wide range of color pattern variation found in the adult female.

There is considerable variation in color pattern. Dunn (1917) found the females from one locality tended to exhibit more of a striped pattern than the males. The limited series of adults at Lantz's disposal were in agreement with Dunn's findings and Lantz (1930a, p. 64) has generalized as follows (translation):

"The original pattern, which may still be distinctly recognized in the female, is formed out of two stripes, which, beginning behind the nostril extend over the eyelids, parotids, neck and sides of the back to the tail. These longitudinal bands, especially on the neck and tail, are connected by a number of cross-bands, which in the female are ordinarily few; in the male, however, they can be so fully developed that nothing more remains of the original longitudinal pattern, in which the cross-bands on the sides of the body and tail either are not further joined or scarcely meet one another."

We have examined over five hundred breeding *A. opacum* and have failed to find any consistent differences in color pattern between the sexes. In figure 84, from among the three hundred and thirteen adult specimens collected on Long Island during the fall of 1931, we have selected individuals of both sexes with the striped and others with the cross-barred pattern predominant. Again, among the eighty-one adult females found guarding their eggs at Oakwood, Virginia, and Rock Creek Park, Washington, during October 1929, we have selected a series which shows nearly the complete range of color variation in the species (Fig. 85). The males collected in the same region fall within this range of variation. Both males and females may be either predominantly striped or cross-barred. We have found no consistent difference between the Long Island and the Washington specimens.

COURTSHIP

Lantz (1930) was the first to describe the spermatophore of *A. opacum*. He found several attached to moss in a terrarium containing several adults. He writes, "As is well known, the marbled salamander breeds in the autumn, and the mating season appears to be quite short. In the two cases observed it was restricted to a few days in the first half of October. During this time unusual agitation prevailed in the terrarium after nightfall. The males were steadily pursuing the female, pushing her and rubbing their snouts against her body. They were also seen to chase each other in very much the same manner. Unfortunately nothing further could be observed, the animals' being so easily disturbed by light that even switching on a weak electric lamp made them soon retire into their hiding places. The actual procedure of fecundation remains, therefore, undisclosed."

Recently it has been emphasized that the evolution of courtship of the Caudata follows closely the phylogenetic scheme (Noble 1931). The courtship behavior remains essentially the same throughout a group of related species in spite of the radically different habitats such species may frequent. Plethodontids, whether aquatic or terrestrial, have the same type of courtship and the same has been shown to be true for certain natural groups of salamandrids. It seemed to us important to determine if *A. opacum*, a terrestrial species, had the same type of courtship found in the aquatic forms of *Ambystoma*.

Methods of Study

Our field observations on courting *A. opacum* were made from September 9 to 15, 1931, in the vicinity of Washington, D. C. These were supplemented by laboratory observations during the same period in Washington. From September 24 to 28, 1931, a series of Long Island specimens were observed under laboratory conditions in New York. Observations in the field were made by means of electric flashlights covered with red cloth. In the laboratory the entire illumination was provided by a series of photographer's red electric light bulbs of only 10 watt capacity.

Many of the salamanders were imprisoned on their courtship grounds by covering them with crystallizing dishes. Others were observed in courtship without such a cover. In the laboratories of the American Museum two or three pairs of adults were placed together in separate crystallizing dishes 20 cms. in diameter. Each dish was provided with several large pieces of damp slate, and was covered. The dishes were placed in shallow troughs of running water and the temperature regulated by the addition of ice. In the laboratories of the National Zoological Park the bottoms of the laboratory tanks were provided with soil from the breeding grounds. The soil was arranged to form either a flat surface or one cut by a series of interconnecting crevices and depressions, to simulate the conditions found in the dry bed of a pond. The soil was moistened to approximate natural conditions. Observations were made in Washington on segregated pairs and on groups of several pairs. Since many adults may be found together under the same log or pile of debris during the courting season, it is probable that group courtship is the rule in nature. However, the movements of individuals seem to be the same whether they are in pairs or in larger groups. Observations in the Washington area were made by Brady, those in the New York area by Noble. Our accounts were drawn up independently and found to agree except in certain details to be noted below. The discrepancies may be due to the fact that many of the Long Island salamanders had courted before reaching the laboratory. Brady, alone, observed the actual acceptance of the spermatophore by the female salamander, with her cloaca. Both of us observed spermatophores deposited by various males.

The Inception of Courtship

In the laboratories of the American Museum six to ten crystallizing dishes of adults were kept under observation at one time. During most of the period the salamanders either remained quiet or moved slowly over the damp stones. On several occasions the beginning of courtship was witnessed. One male would suddenly begin to dash rapidly about the dish butting his head against any individual he chanced to meet. This surprising activity would stir up the group and often another male would begin the same butting performance. A close examination of these active males revealed that they were usually endeavoring to push their snouts under individuals they chanced to meet. Their efforts were directed as forcefully towards other males as towards females. For example on September 24 at 8:45 P. M., one male was seen to butt his snout twelve times in rapid succession against the inguinal region of another individual which was proved by cloacal examination to be a male.

A female was never observed to begin a courtship and when butted by a courting male she exhibited less response than a male under the same circumstances. In attacking the tail of another salamander the courting male attempts to lift up the appendage with his snout but he usually strikes the tail in lowering his head after each lifting movement, thus giving it another rub. On several occasions a quiescent female was observed to arch her tail when this organ was butted by a male. This sometimes resulted in the male's working his way under the tail, or by turning he frequently crawled under the body. When two courting males met head on each tried to thrust his head under the chin of his opponent. The moment one male touched the chin of the opposite male with the top of his snout the opponent would jerk his head to the side and bring it with a vigorous thrust back under the chin of the first. The result was a head "fencing" conducted with such speed that the two heads appeared a blurr. To one familiar only with the slow movements of *A. opacum* outside of the breeding season, this show of agility was a startling performance. The behavior was witnessed three times on September 24, once on September 27, but has not been seen in the field.

Courting males may show a greater interest in other males than in females. On September 24 one male, after methodically thrusting his snout into the sides of a female for over a minute, turned toward a male and continued the butting movements with much greater vigor. Again on September 28 at 8:30 P. M. a courting male met another male and a female head on. The courting male rubbed the side of his head first against the male, and then against the female. He then turned and gave his full attentions to the male.

Lantz, as indicated above, found that courting males would pursue other males, and we have both confirmed this observation many times. This failure to discriminate between the sexes at the outset of courtship results in an increase in general excitement throughout the group. It has a mutually stimulating effect and brings more males into courtship at one time than would otherwise occur.

Courtship Postures

In addition to the butting and prying reactions there were several other types of behavior pattern which appeared many times both in the laboratory and in the field groups. If a butted individual attempts to escape, the courting male will frequently throw his hind legs and tail directly across the path of retreat (Fig. 86). The courting male with his body thus curled will usually continue to thrust his head into the side of the annoyed individual. The position is assumed not merely to trap the escaping salamander but to stimulate it further. With every head thrust the male will often jerk his pelvic region toward the snout of the pursued salamander, with the result that the latter receives almost simultaneously a push in the side and a slap across the snout. On September 28, one male was observed to butt a female nine times with his head while maintaining this position and jerking his pelvis toward her at each thrust.

In the field it appeared that the male was primarily interested in thrusting his head under the cloaca of the female. In the laboratory, however, males which had succeeded in assuming this position frequently worked forward under abdomen and chest, vigorously pushing up with the top of the head. If the

male had thrust his head under the fore part of a female he would frequently work back with the same lifting movements to the cloaca and beyond along the tail. Such movements often result in the female's being lifted from the ground and thrown to one side of the active male. Lateral rubbing movements of the
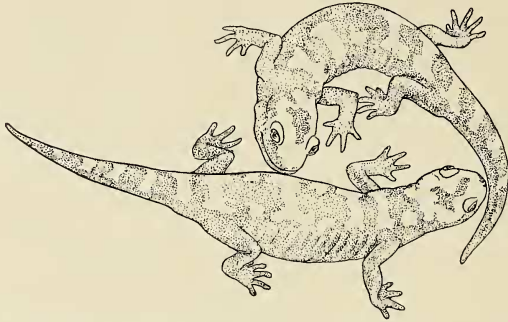

Fig. 86. Courtship of the marbled salamander. While butting the female in the side with his snout, the male blocks with his tail her escape.

male's head may occur when he has thrust it under another individual. The lifting thrust may be accompanied also by side movements of the head. During the less active moments of the courtship the male may rub his cheek across the cheek of another individual. Females have been observed to respond in the
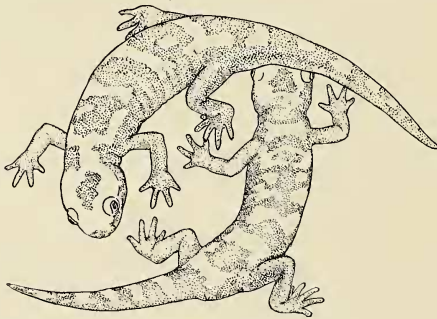

Fig. 87. "The waltz" in the courtship of the marbled salamander. The male (right) attempts to thrust his snout under the tail base of the female while the latter endeavors to bring her snout in contact with his cloaca. The result is a rapid circular movement of the pair.

same manner. The female exhibits her rising interest in the attentions of the male by ceasing her attempts to escape and often by turning her head toward the male. In the field and in the laboratories of the Zoological Park it was noticed that the female frequently turned her head in the direction of the male's cloaca and attempted to put her snout in contact with it. This resulted in

bringing her own cloaca in front of the male's head. The male moving forward to reach the female's cloaca would withdraw his own and the movement continued would result in a circling "waltz" (Fig. 87).

In the laboratory, pairs of males were often seen to circle around in a "waltz," each attempting to thrust his head under the tail or cloaca of the opposite one. In Washington it was observed that if the waltzing salamanders were both males the movement did not continue very long. Only twice was it found to exceed sixty seconds, while if the partners were of opposite sex it would frequently continue for several minutes. It seems probable that cloacal secretions of the female stimulate the male to continue the "waltz" for these extended periods.

Although a male usually attempts to crawl under a partner, he may scramble over. This is generally the case if he does not succeed in thrusting his head under one end of the other individual, in which event he sometimes scrambles over his partner to the opposite end. Although such movements are accompanied by more or less butting with the head, there is never a definite attempt to cover up the nostrils of his partner in the manner characteristic of plethodontids (Noble and Brady, 1930a). However, in the course of a scramble a male may bring his chin over the snout of a female. Sections of this chin skin show that no hedonic glands (cf. Noble, 1931, Fig. 49) are present. Hence the stimulus of this phase of the courtship is mechanical rather than chemical.

The Taking-up of the Spermatophore

We expected to see the spermatophore produced at the climax of the courtship. This was not always the case. For example, on September 27 at 7:45 P. M. the following was recorded:

"Male slowly approaches another male and undulates the base of his tail. He butts side of the male and then turns to a female, slowly rubbing the upper portion of her side, and works towards her tail. During this procedure the male's tail is undulating rapidly. When he reaches the female's tail, he raises his body on legs held stiff and straight and slowly extrudes a spermatophore. Then the male turns and butts the opposite side of the female. After a moment's rest the male turns to another male and butts him with far more vigor than he displayed with the female. A second female passing attracts his attention and he continues vigorous thrusting against her."

The undulation of the base of the tail invariably preceded the production of a spermatophore, although such tail movements also occurred at other times. The undulation sometimes ceased immediately after the spermatophore was deposited, but in other cases it was continued for a few seconds. In seventeen instances the undulation continued until a second spermatophore was produced. One male was recorded as producing three spermatophores one immediately after the other. In six of the eight occasions when spermatophores were taken up by the females of isolated pairs the acceptance occurred immediately following production. The female, moving directly after the male (Fig. 88), passed her chin and body over the spermatophore until the cloacal lips came in contact with it. In the other two cases, several seconds of nosing around transpired before the female snout came in contact with the spermatophore. Then she

moved her cloaca over it. In five of these acceptances the female was seen to undulate her pelvic region while her cloaca was directly over the spermatophore. In three other cases where the spermatophore was secured by the female, she was found to remain motionless over the spermatophore for several seconds. In
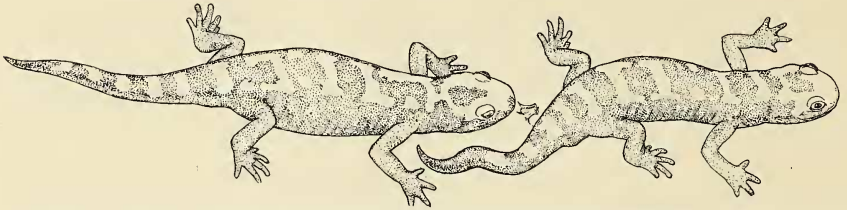

Fig. 88.   The deposition of the spermatophore.   In the final stage of the courtship of the marbled salamander the male deposits the spermatophore while the female is following directly after him.   If the female fails to secure the spermatophore head with her cloacal lips she may nose about until she has found it.

one instance, the female, after covering the spermatophore a few seconds, moved a few centimeters away in the direction of the male. She then turned, and apparently smelling the floor, worked her way back to the spermatophore and resumed her position over it.

As previously stated, group courtship appears to be the rule in *A. opacum* because many pairs were found together. In several groups studied, both in the field and in the laboratory, some or all of the males involved produced spermatophores. The sufficiently stimulated female usually accepted the first spermatophore with which she came in contact. This was usually the one produced by the male whose cloaca she had been nosing. In twenty-eight recorded instances the female apparently became aware of the spermatophore by smelling it, since her snout was brought in contact with it before she moved over it. However, both sexes appear to nose the ground frequently while courting. In six clearly seen cases and apparently in many others, the female first reacted to the spermatophore when her cloaca came in contact with it. In either event the female remained with her cloaca over the spermatophore for several seconds, the longest recorded instance lasting nearly one minute. Usually, however, when a number of individuals were involved, the female would be forced from her position after a short interval. In such instances the female frequently sought contact with another spermatophore. One female thus covered five spermatophores. The taking up of the spermatophore followed most quickly when the female was following the male or moving with the male along the corridors of crevices. One typical record will serve to show how both methods of taking up the spermatophore may occur in the same group. The record was made at the lower pond in Dead Run Swamp, on the Virginia shore of the Potomac, opposite Plummer's Island.

Record of Courtship

"The female first moves away from the spermatophore which the male has produced, although she has been following him for some centimeters. She

later moves back to cover it and shows definite undulations of the base of tail, less intense than the tail wag of the male. A male is now following a female and is rubbing her with his chin. The male wags his tail while following female and rubbing her. The male stops female by throwing tail across her path. The male is now astride female and rubs her with chin. Female slides back and follows male with nose near cloaca. Pair separates. Two spermatophores found on floor. Pair in full waltz. Male crawls over female and rubs her cloaca. Female is now following male. He undulates his tail base and produces a spermatophore. The female misses it and noses about on the ground 5 centimeters from the spermatophore. She finds it and moves first head, then body, and finally cloaca over it. The female undulates her pelvic region. Male still active and turns to female on side of dish. The male thrusts his head under female's cloaca and raises her from floor. Female follows male but no spermatophore is produced. Male now follows female, his tail undulating. A spermatophore is produced but the female turns toward male and the pair waltz. Both undulate their tails. Male plainly seen to produce a spermatophore. The female in waltz position stops, moves away from spermatophore. She turns and moves over spermatophore and secures it while undulating tail base.''

Many other records were made in the field, both with glass covered and with exposed salamanders. At Ritchie, Virginia, in a pond where many courtships were recorded, the temperature was 26°–26.5° C. At Dead Run Swamp, Virginia, the temperature ranged from 23.3°–25° C. These temperatures were higher than those maintained in the laboratory.

Many spermatophores were damaged by the adults' walking over them. It was difficult to be sure even under ideal laboratory conditions how much of the spermatophore was taken up by the female with her cloacal lips. Certainly in most cases only the head of the spermatophore was removed by the female. In
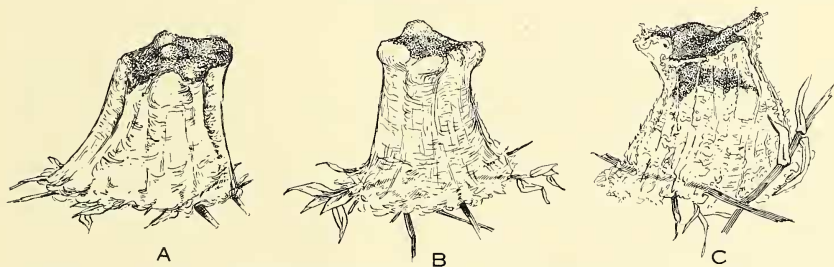
Fig. 89. Spermatophores of the marbled salamander, × 4. A. Freshly deposited spermatophore. B. Spermatophore from which most of the head has been rubbed off. C. Compound spermatophore.

Washington, in only five of the fifty or more cases of impregnation observed were the females seen to envelope the entire spermatophore with the cloaca and remove it. In at least twelve the female removed all or part of the head, leaving the stalk still adhering to its place of deposition. Spermatophores usually were deposited on a fairly loose surface of crumbling leaf mold, hence in most cases

the female dragged the stalk from its place of attachment before she succeeded in removing the head. In New York where wet slate was provided, headless spermatophores were frequently found firmly glued to the rock. In the melée of courtship many spermatophores are doubtless crushed and in nature many are presumably covered with dirt or bits of debris, rendering them difficult to see.

The Spermatophore

A typical spermatophore of *A. opacum* is shown in Figure 89. It measures 4 to 5.5 mm. in height and its stalk is approximately 2 mm. wide at the apex and 6 mm. wide at the base. It differs from the spermatophore of *Ambystoma maculatum* in being smaller and in having a quadrangular or pentangular summit. Each corner of the summit is formed by a low rounded prominence and the top of the structure is truncate and slightly concave. In the fresh spermatophore these summits are usually covered with a mass of sperm which are thus placed in a decidedly advantageous position for being engulfed by the cloaca of the female. Lantz (1930, Fig. a) has described a spermatophore which seems to have lost most of this sperm mass. A fresh spermatophore is less concave at its summit than the one he figured, and there is usually a thin cap of white spermatic fluid completely covering the truncate top. The central portion of this surface may be raised into a slight eminence. Sections reveal that this central prominence is formed chiefly of eosinophilic granular substance which agrees in character and staining properties with the secretion of the male's pelvic gland. The spermatozoa are spread over the top of the spermatophore. A remarkable feature is that the heads of the spermatozoa are often all directed the same way. Thus in the micro-photograph (Fig. 90) of the head of one of the spermatophores it may be noticed that the spermatozoa covering one of the four summits are directed outward. This orientation of the spermatozoa would appear to be advantageous to a rapid impregnation.

The base of the spermatophore appears glassy, but slightly frosted, to the unaided eye. Sections reveal that the basophilic mucous material of this base is divided up into short segments by thin partitions of eosinophilic material. Unlike the eosinophilic material on the spermatophore head, this is not granular but homogenous. Sections of the cloaca of the male *A. opacum* reveal that this eosinophilic scaffolding of the stalk is produced by a series of glands lying on either side of the pelvic gland in the roof of the cloaca. The secretion of these glands is homogenous, in sharp contrast to the secretion of the pelvic glands. Unlike the cloacal glands which line the walls of the cloaca, this secretion is not mucus. With thionin, methylene blue or haematoxylin the secretion of the cloacal glands is stained blue. The abdominal glands, which empty on the cloacal lips distal to the cloacal glands, produce an eosinophilic secretion which is finely granular and hence readily distinguished from both the pelvic gland and from the gland which produces the eosinophilic scaffolding of the stalk.

The abdominal gland may pour its secretion directly to the outside when the cloaca is turgid. It has been assumed that in aquatic salamanders which drive a current of water towards the female during courtship, the abdominal gland secretion is carried in this current and tends to stimulate her. It seemed

to us possible that it was the secretion of the abdominal gland which chiefly interested the female during the "waltz" phase of the courtship. The question arose as to whether or not the spermatophores on being produced become covered with a layer of abdominal gland secretion which would serve to make them, like the male cloaca, attractive to the female. We have shown above that the spermatophore is found by the female even when not in line with her body.
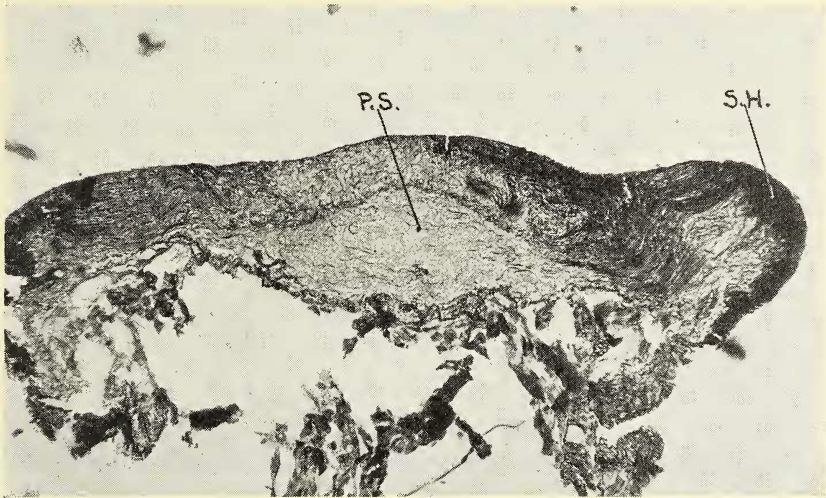

Fig. 90.   Vertical section of the spermatophore head of the marbled salamander, × 61.
P. S. Pelvic gland secretion.   S. H. Mass of sperm heads.

Our sections of the spermatophore fail to reveal evidence of a covering layer of abdominal gland secretion. Moreover, in our sections of the cloaca of the male the secretion of the pelvic, scaffolding, and cloacal glands may be seen in the lumen of the cloaca, but no abdominal gland secretion is found there. If the abdominal gland plays any part in spermatophore formation it produces at most a very superficial covering.

Lastly, it may be pointed out that *A. opacum*, like other species of *Ambystoma*, exhibits some variation in the form of the spermatophore. The groove which is found on one side of the typical spermatophore is frequently lacking. Again, double spermatophores (Fig. 89C) may be produced. Smith (1910) has described compound spermatophores in *A. maculatum*. We have not found a compound spermatophore formed of more than two spermatophores in *A. opacum*.

Method of Sex Recognition

From the above description it may be concluded that there is no recognition of sex at the beginning of courtship but that males endeavor to stimulate any salamander of their own species they chance to meet.

In *Ambystoma maculatum* the males engage in a Liebesspiel with other males and attempt to push under them. Wright and Allen (1909, p. 690) described the action of a male toward a female:

"It seemed to be the object of the male to bring the top of his head in contact with the venter of the female. The throat region of the female seemed to be preferred, although he often began in the cloacal region or even at the tip of the tail and rubbed the dorso-lateral part of the head along her whole ventral side." During the Liebesspiel of *A. maculatum* it has been noticed that the males respond in exactly the same way to other males (Noble 1927, p. 34). Wright and Allen found that the mere presence of females in jars with the males stimulated the latter to the production of spermatophores. We have noted in *A. opacum* that spermatophores are deposited more often after a male has been rubbing a female than at any other time. Spermatophores usually were not produced at the moment the male was nosing the female's cloaca, and it seems probable that secretions from the general integument as well as from the cloaca may excite the males. It may be concluded that in both *A. maculatum* and *A. opacum* the males become sexually active sooner than the females. They excite the colony to sexual activity by engaging in a series of rubbing movements which are exactly the same whether directed toward male or female. Eventually the odor of the female excites the male to the production of a spermatophore. The antics of the male induce the female to follow him and this may automatically lead to the picking up of the spermatophore. However, our work has also disclosed that the female shows a definite interest in the spermatophore and when her body is not in line with it she will move toward it for the purpose of covering it. At the height of the courtship the female appears to be interested in bringing her snout close to the cloaca of the male. The orifice of his cloaca is provided with a series of abdominal glands which are believed to produce a secretion stimulating to the female. It has been suggested above that the abdominal glands may coat the spermatophore with a secretion which makes it attractive to the female. We could find no histological evidence in support of this assumption. Hence it is possible that the other secretions which enter into the formation of the spermatophore are odorous and attractive to the courting female. In brief, our observations suggest that secretions of the integument and cloaca play an important role in sex recognition and stimulation. In this *A. opacum* agrees with the other species of *Ambystoma*.

COMPARISON WITH OTHER SALAMANDERS

As indicated above, the courtship of *A. opacum* agrees essentially with that of *A. maculatum* even though the first proceeds on land and the second in the water. This is further evidence in support of the thesis (Noble 1931) that the courtship pattern is relatively stable in evolution. However, the only other species of *Ambystoma* whose courtship is known in detail, differs markedly from *A. opacum* both in the ability of the males to distinguish sex and in the courtship pattern. Until recently there has been some uncertainty as to the courtship of this species, *A. jeffersonianum*, because the original describer reported the males to embrace "exactly in the manner of the spotted newt" (Wright 1908), and

subsequent observations (Mohr 1931) showed that the male gripped with his forelimbs and usually not with his hind. The observations of Mohr have been confirmed and extended by Kumpf and Yeaton (1932) and one of us has been able to witness the performance several times. Kumpf and Yeaton recorded the courtship of two male and three female *A. jeffersonianum*. There is no doubt that, although these males gripped one another at times, they could distinguish the opposite sex far more rapidly than any courting specimens of *A. opacum* which we observed. The courtship pattern resembled that of *Triturus pyrrhogaster* or *T. torosus* far more closely than it did that of *A. maculatum* or of *A. opacum*. Mohr (1931) concludes "that there is no marked difference in the courtship behavior of *A. maculatum* and *A. jeffersonianum*." The evidence he has so far presented in favor of this is brief. He states that a single male "was seen to be vigorously undulating his tail and whole posterior part of his body. Several times he clutched convulsively at the ground with his hind legs. A female approached, nosing the cloacal region of the male, which almost immediately deposited a spermatophore. The male crawled very slowly away, undulating his tail feebly. The female followed and came to rest with the cloaca immediately above the spermatophore and remained motionless for perhaps fifteen seconds, then pushed vigorously after the male into deeper water."

A second male in the laboratory "swam over a female, grasping her with the forelegs, then slid slowly backward and forward rubbing his cloacal region over the dorsal pelvic region of the female."

At the present time we have no evidence that the male of *A. jeffersonianum* attempts to pry under and lift up the female during courtship, nor is there evidence of a Liebesspiel engaged in by males alone before the females have become stimulated. There are various rubbing movements, but apparently these are not specific for *Ambystoma*. On the other hand the forelimb amplexus is a new phase in *Ambystoma* courtship, a phase which is further developed in another group of salamanders. It has been suggested (Noble 1931) that the courtship of *Ambystoma* is sufficiently generalized to form the ground plan out of which the courtships of the higher groups of urodeles may be evolved. The observations of Mohr and of Kumpf and Yeaton lend support to this view. Their work has also shown that there has been an extraordinary evolution of the pattern of courtship within the genus *Ambystoma*. Further, one species has developed a courtship pattern which is essentially the same as that of certain primitive salamandrids. Since two species of *Ambystoma* have a simpler type of courtship in the sense that sex discrimination is made less rapidly it would follow that the courtship pattern of *A. jeffersonianum* has evolved out of that of these two species. *Triturus torosus* and *T. pyrrhogaster* are not close relatives of *A. jeffersonianum*. Hence the courtship pattern of *A. jeffersonianum* would appear to represent a case of parallel evolution. Many cases of the development of the same structural modification in unrelated groups have been described (Noble 1931). The evidence at hand appears to indicate that the courtship of *A. jeffersonianum* represents a parallelism rather than a case of true genetic affinity.

It may be noted, however, that *A. jeffersonianum* agrees with *A. opacum* in the smaller number of spermatophores produced. This is correlated with the fact that in neither species do the males congregate in great numbers and engage

in a Liebesspiel of several hundred individuals. Our field observations indicate that such large assemblages are characteristic of *A. maculatum*. Because of the irregularities of the terrain these mass formations could not be carried on with any success in *A. opacum*. Possibly the smaller number of courting *A. jeffersonianum* is correlated with the rarity of the species in any one locality. However, Mohr (1930) has recorded 200 specimens of this species in one locality. He states (p. 53): "Everywhere, within three or four feet of the shore Jefferson's salamanders were rising to the top, taking a bubble of air after the fashion of the spotted salamander. Close to shore the salamanders seemed to be congregating." This description agrees closely with our observations of the Liebesspiel of *A. maculatum*. Mohr remarks that the spermatophores of *A. jeffersonianum* are less conspicuous than those of *A. maculatum*. Possibly this is the reason they are not found in large numbers.

In both *A. opacum* and *A. jeffersonianum* there is a smaller mass of spermatozoa in each spermatophore than occurs in *A. maculatum*. This would appear to be correlated with the fact that both species produce on the average fewer eggs than does *A. maculatum*. The spermatophore of *Triturus viridescens* has a very small head of sperm and this species, like *A. opacum*, may lay only half as many eggs as *A. maculatum*. However, to judge from Smith's summary (1911), the difference between the average egg number in the species of *Ambystoma* is not as great as the difference in sperm head sizes. Until a study is made of the number of spermatozoa in the sperm heads of these spermatophores, it will be impossible to state how close is the correlation between egg number and spermatozoa number in the various species of *Ambystoma*.

The Spermatozoön

Lantz (1930) appears to have been the first to describe the spermatozoön of *A. opacum*. He states (p. 323):

"At high magnification the sperm looked a tangled mass of spermatozoa intermingled with numerous other much longer and slightly thinner filaments. The spermatozoa are about 75 μ long, thread-like, extremely thin and bear a narrow pointed head piece about 6 μ long. At the time the observation took place they were all perfectly inert, and curved into semicircular shape."

We have examined sections of spermatophores fixed in Zenker's fluid (bichromate of potassium and corrosive sublimate) and have also examined smear preparations of spermatophore heads fixed in Bouin's and in Zenker's solution. We have also examined teased preparations of the fresh spermatophore head. The latter proved much less satisfactory than the fixed material. Delafield's haematoxylin and eosin were used for stains.

The spermatozoa of *A. opacum* are very different from the structures Lantz (1930) has described and figured. They closely resemble the spermatozoa of *Ambystoma mexicanum* as figured by Retzius (1906). The tail is provided with an undulatory membrane extending the entire length except for the tip, which is provided with a lash or end piece. Opposite the membrane a flat keel (Fig. 91) extends for approximately one-sixth of the length of the tail. A similar but apparently shorter keel occurs in *A. mexicanum* and readily distinguishes the
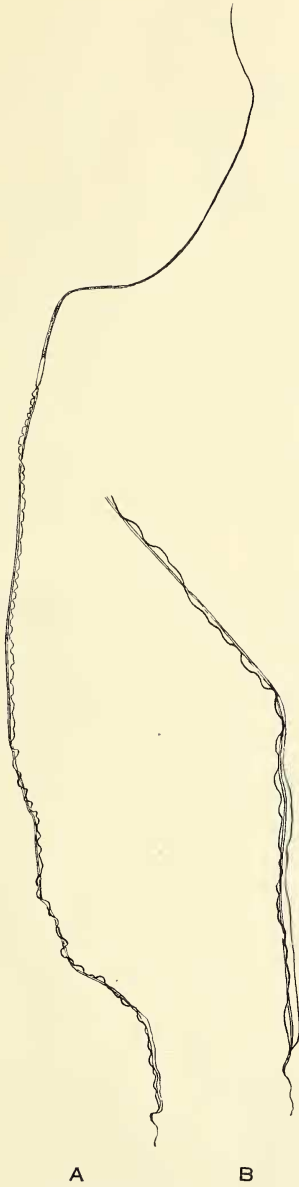
Fig. 91. Spermatozoön of *Ambystoma opacum.* A. The whole spermatozoön viewed laterally. B. The caudal portion, under higher magnification showing the forward extension of the keel.

spermatozoön from that of the plethodontids so far as is known (Noble and Weber 1929).

The spermatozoa of *A. opacum* differ from those of *A. mexicanum* as figured by Retzius (1906) in their shorter lash on the tail, the longer head without a barb, and in the less protruding centriole of the middle piece. Under oil immersion the head of the spermatozoön is found to taper to a very fine point. In some preparations the delicate point, or acrosome, may be bent over, but we have failed to find a distinct barb present. This is the more surprising in that the barb is found on the acrosome of many other species of salamanders (Wilson 1925). In our stained preparations the middle piece has a very distinctive appearance. Its anterior fifth or at least the margins of this region take a deep blue stain in striking contrast to the remainder of the middle piece, which stains a bright pink. The spermatozoa vary somewhat in length. Well-fixed spermatozoa are more than six times as long as the structures Lantz described. We have failed to find the "numerous other much longer and slightly thinner filaments" unless these be represented by the sperm heads which, to judge from Lantz's figure, he apparently did not see. Spermatozoa in our smear preparations vary from 480 μ to 530 μ in total length. The parts of a typical spermatozoön measure as follows: head 150 μ, middle piece 10.2 μ, tail exclusive of lash 320 μ, lash or end piece 7.5 μ.

The Egg

There is a general agreement among the various writers as to the number of eggs laid by *A. opacum*. Mann (1855) reported 108 eggs; McAtee (1907) 50 to more than 150; Dunn (1917) over 100; Bishop (1924) two sets of 102 and 73 eggs; Lantz (1930) 94 the first year, 103 the next, for his laboratory specimen. Fifteen clutches taken at Oakwood, all of which we are reasonably certain are single sets, give the following figures: 75, 81, 101, 122, 125, 132, 139, 143, 147, 167, 173, 191, 193, 226, 232, resulting in an average of approximately 150 for the typical sets. We found one set at Oakwood which contained 340 eggs. Since this nest was guarded by but one female and was somewhat off from the main nesting area, it may have been a single set. This number is so large, however, that we do not include it in our average. In a great number of cases in the principal nesting area, as has been stated above, it was not possible to tell where one set began and another ended. In addition to the close proximity of the nesting depressions, as a factor in bringing this situation about, the females apparently move along the tunnels during oviposition, which results in the stringing out of many clutches. A number of eggs apparently had rolled away from the main portion of the sets, for eggs would be found scattered singly or in little groups at various points along the principal nesting tunnels.

Measurements of the eggs with their capsules in the field ranged from 2 to 5 mm. according to the degree of moisture to which they had been exposed. It was subsequently determined in the laboratory that the egg was capable of desiccation to a point where it measured approximately 1.5 mm. without injurious effect. Two batches of 50 eggs each, in this condition when found in the field, weighed 7.2 gms. and 6.68 gms. After twelve hours immersal in water they had gained 5.2 gms. and 6.84 gms. respectively. In another twelve hours

they had gained 2.2 and 1.48 gms., making their final weight 14.6 and 15 gms. respectively. After this there was no further increase in weight. One Oakwood clutch of 125 eggs was divided into five equal portions which weighed, separately: 3.2, 2.75, 4.5, 3.4, 3.92 grams. After twenty-four hours in water their respective weights were 7.2, 6.5, 7.38, 6.5, 6.75 grams. A clutch of 154 eggs from Rock Creek Park weighed 21 grams as taken from the nest. In twenty-four hours immersion it increased 17.75 grams in weight. Another twenty-four hours in the water increased its weight 1.5 grams. From this it will be seen that the eggs, in their normal condition as found in the nest, are capable of absorbing approximately their own weight in water, during the first twenty-four hours immersion. After that period the rate of absorption drops to a negligible point.

Although the eggs are normally deposited separately, we encountered several instances in which a cluster of several eggs were stuck together, adhering to each other by their sticky outer coverings. We also recorded several instances of two eggs' being connected by a well-developed pedestal. In two instances two eggs were enclosed in a common envelop.

The egg of *A. opacum* has been described as being enclosed by two envelops, an outer and an inner, separated by a jelly-like layer (Bishop 1924). Lantz (1930, p. 323) states:

"The egg is spherical, 4 to 5 mm. in diameter and possesses two membranous envelops, separated by a coat of jelly. A much less viscous medium surrounds the vitelline sphere, which measures 2.5 mm. in diameter."

The egg of *A. opacum* preserved in formol appears to have four capsules: a clear wrinkled outer capsule, a grayer and thicker capsule immediately within this, a clearer capsule of the same or slightly greater thickness coming next in order, and finally a thin but highly refractive capsule forming the innermost of the series. Between the inner capsule and the egg with its closely adherent vitelline membrane is a large water space. The vitelline membrane, often difficult to see in living eggs, is clearly visible in these eggs when a cleavage furrow is viewed from the side. A typical egg measures 2.8 mm. in diameter. The greatest diameter of the water space is 4 mm. The diameter of the surrounding envelops, reading from within out, is as follows: 4.1, 4.5, 4.8 and 5 mm.

In the fresh eggs these same capsules may be recognized readily even in those four months old and ready to hatch. The outer capsule is thin and soft. Dirt and other debris adhere to it. Within this is the thicker and resistant second capsule. When this is cut through, the embryo, enclosed in the highly refractive inner capsule and the one surrounding that, slips readily through the puncture. It is then seen that the capsule third from the outside is very soft and gelatinous as Bishop and Lantz state. We prefer to call this a capsule rather than a jelly layer since it has the structure of a capsule in eggs preserved in formol. In living eggs it differs from a capsule in that it forms an amorphous mass when pulled free from the other capsules. In fresh eggs ready to hatch the water space surrounding the vitelline membrane is eliminated by the pressure of the large embryo. In brief, we recognize the same structures Lantz described in the egg of *opacum* but we call his "coat of jelly" an egg capsule and we recognize an additional outer capsule formed of soft adhesive material.

The Brooding Habit

Much has been said of the attitude of the female *A. opacum* toward her eggs. It is generally agreed that an adult is usually associated with the egg mass and the guardian upon examination has been found to be a female. In 1929, we collected eighty-one females guarding their eggs. Only one male was taken in the nesting area. It was found near a female with three freshly laid eggs in a very indistinctly formed depression under a brush pile. This case may indicate that, in nature, oviposition closely follows impregnation. Lantz (1930) found that oviposition did not occur until two weeks after fecundation in his laboratory specimens. We have seen spermatophores produced the same night that eggs were laid in the laboratory (September 28). Only one female was found outside the principal nesting area at Oakwood during 1929. This was a spent animal and was not associated with any eggs although it is possible that her nest had been overlooked. In some instances it was not possible to identify the respective broods of adjacent females, the clutches being so confluent that we were unable to separate them. In several instances nests were found unattended, in both the Oakwood and Rock Creek sites, and at another point nearer Oakwood. It is quite possible that the disturbed females in these cases made off before discovery. As will be pointed out below, the female will usually desert the eggs upon being disturbed.

Mann (1855) describes the female as being found "curled up" on top of the eggs. Dunn (1917) reports the mother as "lying on top of them." Lantz (1930) writes the "female sits on the eggs." In our experience the female was usually found on the periphery of the egg mass, the tail either turned toward and encircling some of the eggs or turned away from them. The clutch itself was invariably too large and too well spread out to permit the female to come into contact with more than a small portion of it at a time. When completely exposed the female usually pushes her snout into the egg mass, a reaction apparently due to negative heliotropism. Often, when a convenient means of escape is at hand, the female departs with celerity.

In the plethodontid salamanders the female will return to her eggs, apparently being attracted by them. Wilder (1917) found that a female *Desmognathus fuscus* will brood the eggs of another female. We have performed some experiments both in the field and in the laboratory to determine how close is the bond between the female *A. opacum* and her eggs. In the field brooding females were marked for identification by ligatures attached to the limbs. Then these females were transferred into one another's nests. In one instance, one of the females remained in the strange nest into which she was placed the day before, while the rightful owner of the nest disappeared from the strange nest into which she had been placed. Marked specimens were removed to distances of from twenty-five centimeters to one meter from their nests. The nests were carefully covered but the next day no female was found to have returned to her nest. In the case of two adjacent nests found unattended the first day, a female was found to have appeared and come into contact with one of the groups of eggs the next day.

In the laboratory a crystallizing dish was filled with clay sub-soil from the

nesting area. Inter-connecting depressions, six in all, corresponding to those in which the nests were found, were made in the clay. A clutch of eggs was placed in one of these depressions. The whole was then covered with a layer of moss and leaf mold, to correspond to the conditions in the nesting area. The female found with this clutch was then placed in the dish. Although she stayed in the depressions during a period of twenty-seven days, she moved from one to another without evincing any preference for the one containing her eggs, but showed a disposition to remain for some time in any one depression.

Four nests were placed in soil and débris in a large tank. The nests were then covered with a layer of moss. The four females to whom these nests belonged, having been marked for identification, were placed in the tank. During a period of two weeks they remained in depressions other than those containing the nests. It was again noted that the animals, once located, showed little disposition to move.

Ten nests were placed in crystallizing dishes. The clutches were placed in the centers of the dishes, surrounded by layers of moss and débris. During a period of thirty days no marked tendency to remain with the egg mass was observed in the female. Depressions in the moss seemed to be as satisfactory as the egg mass for the female. The coiled attitude, observed in females associated with their eggs, may also be assumed by the animals when occupying positions removed from the eggs.

From these experiments it would seem that the eggs lack any great attraction for the female *A. opacum* and that definite orientation toward the eggs, such as is exemplified by *Desmognathus* (Noble and Evans 1932), is lacking in this ambystomid. In the laboratory while these experiments were being carried on we had a brooding *Desmognathus fuscus* and a *Plethodon cinereus* which would invariably return to their eggs. Under the conditions imposed in the laboratory there was no doubt that the female *A. opacum* was not attracted by her eggs as strongly as the terrestrial plethodontids mentioned were attracted by theirs.

It seemed to us possible that the disturbance caused by transporting the salamanders might have prevented the normal appearance of a brooding instinct in the laboratory. Therefore, we decided to secure further data on the duration of the brooding period under natural conditions. In the Washington area during 1931 we secured the following data:

In Rock Creek Park on October 12 most nests in the upper part of pond, where it was damp, were found to be deserted. In the lower pond area, where it was drier, some females were found with eggs. Four nests with females were located under a large log pile. One could view these without disturbing them by moving the top log. The temperature on this day was 10° C. There was only a trace of water in the pond but the ground was damp. On October 14, with the temperature at 17.5° C., a slight drizzle falling and making the ground wet, only two of the females were present. We shall call these No. 1 and No. 4; the missing two, No. 2 and No. 3. On October 15, with the temperature at 17.8° C., the rain falling and the ground very wet, all females were guarding their nests except No. 2. On October 16, with the temperature 11.5° C., the rain falling and the ground under the logs wet, females No. 3 and 4 were present but the others were not seen. On October 17, with the temperature at 10° C. and the

ground saturated, all females had left their nests. On October 21, although the ground was dry and the temperature up to 11.2° C., the nests were still deserted. Hatching was in full sway on November 21 when the ground was again very wet. It had been raining hard during the week. On November 22, only eight unhatched eggs were left in nest No. 1, only fifteen in nest No. 2 and forty-three in No. 3.

We made less extensive observations in other localities. For example at a pond on the Glover estate, Georgetown, D. C., on October 4, the temperature was 24° C., and the ground was very dry. Out of fifteen nests seen in one locality and seven in another, only two lacked an attending female. These observations agree with those made during 1929. During early October when the ground is dry the nests are usually attended by the maternal parent.

In Dead Run Swamp on October 11, the temperature was 16° C. and the ground was damp because of extensive rains during the previous week. Most of the nests found were deserted and contained swollen eggs. Only three females were found with eggs near one pond and only one nest with brooding parent near the other pond. Three nests were selected for further observation. No. 1 contained 150 eggs, No. 2 had 225 and No. 3 held 170 eggs. Larvæ could be seen within the egg capsules and a trace of a balancer was observed in specimens examined. This locality was not visited again until October 18. Then the temperature averaged 10° C. No females could be found near their nests. All eggs were found to be greatly swollen. The larvæ examined had absorbed their balancers and some had hatched. In set No. 1 about seven eggs had hatched, in set No. 2 about three. Only nest No. 3, which was on drier ground, had no hatched eggs.

It was very interesting to find that certain salamanders had been feeding on the eggs. Two *Desmognathus fuscus* and no less than five *Eurycea bislineata* were caught very near the eggs. The *Desmognathus* stomachs contained *opacum* eggs and larvæ. Three capsules were taken from the first *Desmognathus* and four capsules and partly digested *opacum* larvæ from the second. Several predacious beetles also were seen with the eggs. This observation indicates that if the female leaves her charge before hatching, the eggs may be devoured by *Desmognathus* and apparently by other enemies.

This conclusion was confirmed by later observation. On November 1, further examination of these nests in Dead Run Swamp was made, when the ground was dry and the temperature was 7.2° C. All three sets of eggs were reduced. Nest No. 1 contained one hundred eggs, No. 2 held only ninety, and No. 3 contained one hundred and twenty eggs. Again Amphibia were captured with *A. opacum* eggs in their stomachs. One *Eurycea bislineata*, two *Desmognathus fuscus* and one *Rana clamitans* were found to have eaten the *opacum* eggs. Many beetles were found in the nests. It does not follow that these amphibian enemies had been attracted to the eggs by smell alone. In the laboratory we have seen a *Desmognathus* snap at an *opacum* larva moving within its egg capsules. On November 15, the egg number was reduced to seventy-five in nest No. 1, fifty-eight in No. 2 and ninety-three in No. 3. Some insects, but no Amphibia, were found in the nests. On November 1 and November 15 a search was made for hatched larvæ in the nests, but none was found.

Unfortunately no opportunity permitted our following the fate of eggs in other localities. These Washington observations indicate:

1.—The female parent leaves her brood about the end of the first month, and apparently after heavy rains.
2.—Before deserting her eggs, the female may move about. Our observations show that she does not guard her eggs continuously.
3.—In the field, as in the laboratory, many eggs hatch on land when the ground is saturated. Probably crevices or crayfish burrows may hold sufficient moisture to permit the survival of these larvæ, which are structurally fitted for life only in water.
4.—When the female leaves, the eggs may be eaten by salamanders, insects or other enemies.
5.—These enemies as well as droughts, considered in another section, play an important role in keeping the species in check.

Lantz (1930) reports that his female *A. opacum* in the laboratory remained with the eggs steadily at first and intermittently later on. We found females in the field brooding their eggs in the daytime and at night. Our field observations indicate that the female may return to the eggs once she has left them. We secured evidence, however, that the females do not forage for food, at least not during the early part of their brooding. We examined the contents of the stomachs of twenty-six brooding females taken in the Washington region in October 1929. Of these nine were empty, and fourteen contained nothing but bits of shed skin and vegetable débris, which, no doubt, had become entangled in the skin at the time of swallowing. The other three contained, besides the cast skin and vegetable matter, (1) an ant and another small hymenopteran, (2) a coleopterous larva, (3) an egg, apparently in early cleavage stages. It seems reasonable to believe that the last item was accidently swallowed and that *A. opacum* brooding females do not feed on their eggs as does the male *Cryptobranchus.*

Lantz (1930, p. 324) remarks in regard to *A. opacum:* "In the natural habitat of this species the eggs are laid in the vicinity of ponds, and the female has been observed to move them to higher grounds when the ponds rose." Our observations in the field lend no support to this assumption. In the Oakwood nesting area we placed a female and her eggs in a mud basin, in a tiny spring pool at the lowest point of the pond bottom. This basin was so arranged that the water partly covered the clutch, but some of the eggs were in contact with the surface. The female was placed on top of the eggs. The next morning she had deserted the nest in the improvised basin. In the Rock Creek station, however, a nest was found partially submerged, the egg capsules being just in contact with the water's surface. The female in this case was sitting on top of the eggs. As we have stated above we have not found this to be the normal position. This nesting site, customarily dry at this time of year, had been flooded by heavy rains which had occurred during the previous week. The instance would indicate that the female does not leave the eggs until full submergence occurs unless she is disturbed. However, as reported above, we have seen other cases of the female's leaving before this time. In most of the nests

we observed there were no lower and upper levels available to which the female could move the eggs and this, coupled with the fact that there would be obvious difficulties in the way of a salamander's moving a batch of one hundred or more separate eggs, makes it seem hardly possible that such behavior ever occurs. The female may play some part in keeping the eggs moist, however. The bladders of all brooding females were found to be greatly distended with fluid and it is quite possible that this factor would be of service in moistening the eggs, as has been suggested in the case of *Aneides lugubris* (Storer 1925). However, the brooding female may have other duties; we never found her nest attacked by the Amphibia or by insects while she was present. Her presence, therefore, may be of some advantage to the eggs.

Development

It is usually assumed from the work of Dunn (1917) and others that the eggs of *A. opacum* can withstand long periods of desiccation and that they do not hatch until covered by the rising waters of the ponds near which they are laid. Lantz (1930, p. 324) has reported some observations which are at variance with this view. He states:

"Considerable latitude exists with regard to the time of hatching and the larvæ will remain alive inside the egg for a very long time if for some reason hatching is delayed. In one egg kept on land the embryo lived 207 days; another which had been immersed in water the day after it had been laid, remained alive four days longer than the previous."

In order to secure more data on the relation of water to growth and to hatching in *A. opacum* we have repeated the experiments of Dunn and of Lantz on large series of eggs. We selected a number of clutches and divided each clutch into three approximately equal parts. One group (a) was placed in water 4 to 5 cm. in depth. A second series (b) was placed on saturated sand while a third series (c) was arranged on dry packed sand in chambers. Covered crystallizing dishes of the same diameter were used for containers in each case. The results of these experiments may be briefly listed:

Experiment 1a.

Forty-two eggs swollen with moisture in the field, placed in water on November 4. In ten days' time 92.5 per cent had hatched, as follows:

November	5	6	7	8	9	10	12	13
Number hatched	7	10	1	3	4	1	6	5

The balancers were large in the larvæ first hatched and reduced in the last. There was little change in the limbs. The first larvæ hatched were 16 mm. in total length, the last 17 mm.

Experiment 2a.

Twenty-five eggs, less swollen than those of Exp. 1a, were placed in water on November 4. In fifteen days 76 per cent had hatched.

November	4	5	6	7	8	10	11	14	19
Number hatched	2	1	7	1	3	1	1	2	1

The balancers were developed in all the larvæ at hatching, but there was

a decided difference in the limbs. In the first hatched the digits of the fore limb were not differentiated and the rear limb rudiments were not visible. In the last hatched three digits were present on the fore limb and the hind limb rudiment was present.

Experiment 3a.

Fifty-six eggs, found in a desiccated state in the nest, were placed in water on November 5. In forty-one days 88.4 per cent of the eggs had hatched:

November–December	5	6	9	13	17	18	23	1	4	6	7	8	9	11
Number hatched	1	1	2	1	1	2	3	11	1	5	7	4	6	1

The first hatched was only 13 mm. in length. It had large balancers and rudimentary fore limbs. The last hatched had the balancers practically gone and three digits visible on the fore limb.

Experiment 4a.

Sixty-three eggs, swollen with moisture from their damp nests, were placed in water on December 4 and within seven days all had hatched.

December	5	6	7	8	9	10	11
Number hatched	7	2	11	7	9	14	13

The first hatched was 20 mm. in length, the last hatched was only 18 long, and had the fore limb digits in a less differentiated stage than the earlier hatched one of larger size. The balancer of the last was well reduced, giving evidence of its greater age. These two larvæ are represented in figures 92 C and D.

Experiment 5a.

Forty-eight eggs, found desiccated in the nest, were placed in water on December 4. In twenty days 75 per cent had hatched:

December	7	8	9	10	11	12	13	19	20	24
Number hatched	1	1	13	7	5	3	3	1	1	1

The first hatched was only 13 mm. long with the fore limbs in the limb bud stage. The lips were barely formed and the balancers were large. The yolk sac was still prominent. The last hatched measured 18.5 mm. in total length. Three digits were present on the fore limb, a rudiment of the hind limb had appeared and the balancers were reduced.

Experiment 6a.

Twenty-eight much desiccated eggs were placed in water on December 4. In thirty-two days all had hatched:

December–January	5	7	8	10	11	12	16	21	22	24	27	28	30	31	5
Number hatched	3	1	1	2	7	1	1	1	3	2	1	2	1	1	1

The hatching of these submerged eggs extended over a period of a month. The enormous difference between the first and last hatched is shown in figures 92 A and B. The first hatched measured 10.5 mm. in total length, the last 18.5 mm. In the first, the lips, the eyes and the gills are rudimentary; the fore limb is represented by a bud. A large yolk sac and a not completely developed balancer are present. In the last hatched the balancer has been absorbed and the other larval structures are well developed.

Experiment 7a.

Thirty-four eggs were immersed in water on December 4. These had been partly dried in the field and were small. Over a period of seventy-six days, only 75.38 per cent of the eggs hatched.

December–January–February	10	11	16	22	2	6	7	8	1	4	24
Number hatched	1	2	3	9	4	3	1	1	1	1	1

The first hatched were only 13 mm. in total length and, like the first hatched in the previous experiment, had rudimentary gills and fore limbs. The last hatched was 19.5 mm. long and had four digits on the fore limb, the hind limb rudiment present, and the gills elongate.

In these experiments eggs frequently failed to hatch. Bishop (1924) found small nematodes emerging from the egg capsules of *A. opacum* simultaneously with the larvæ. He points out that while these parasites seemed to have no effect on the hatched larvæ, they may have accounted for the death of some eggs that failed to hatch. No nematodes were noted in our experiments. A dead egg very soon molds, and mold did not grow on most of the eggs until all the larvæ reported above had hatched. This indicates that the death of the larvæ was due to failure to hatch.

Several conclusions may be drawn from these results:

(1) The development of the encapsulated larvæ is correlated with the degree of moisture to which the eggs are exposed during the time they are in the nest. Swollen eggs usually contain well developed larvæ; desiccated eggs hatch out larvæ in a more embryonic condition.

(2) The time required for hatching, after immersion in water, is variable, ranging from seven to seventy-six days for these seven clutches. Again, the eggs which had been swollen with moisture require the least time in which to hatch.

(3) The percentage of eggs which normally hatch from each clutch is variable, ranging in these cases from 75 to 100 per cent and averaging about 86.75 per cent. In the case of those eggs which failed to hatch, there was always a normal development for a certain period.

(4) The larvæ may emerge from the egg in any one of a wide range of developmental stages.

Dunn (1917) reports balancers and fore limbs on his newly hatched larvæ. Bishop's (1924) specimens all lost their balancers before hatching. Lantz (1930) reports balancers present on his larvæ at hatching, also digits on the fore limb and a rudiment of the hind limb visible. He says, however, that if hatching is retarded the larvæ may emerge from the egg without the balancers and with a rudimentary fourth finger. The total lengths given for hatching specimens [15 mm. (⅝ inch), Deckert, 1916; 19 mm., Brimley, 1920; 15–19.5 mm., Lantz, 1930] do not fully cover the range exhibited by our series. Our specimens at hatching range from very early larval stages in which the eyes were not yet functional, the lips barely formed, the gills still without rami, and the fore limb in a rudimentary bud stage, to advanced stages in which the balancers had entirely disappeared, the four digits of the fore limb had become differentiated,

the rear limb bud had appeared and the larva had assumed a mature larval habitus. The escaping larvæ ranged from 10.5 to more than 20 mm. in length.

In the experiments comprising series "b," as stated above, the eggs were placed on saturated sand, in moist chambers. The sand was banked at an angle and a small amount of water was kept at the bottom of the slope to in-
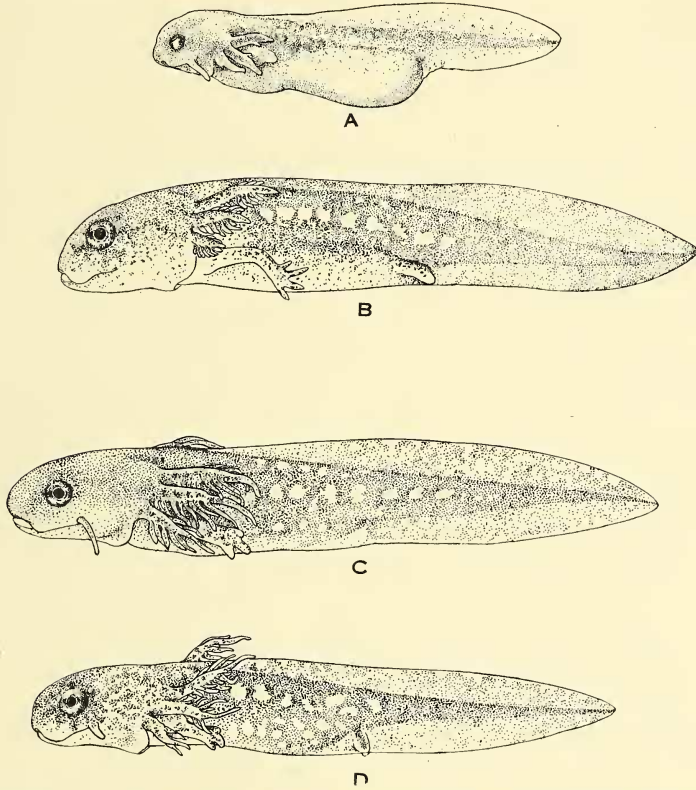

Fig. 92. Larvæ of *Ambystoma opacum* at the time of hatching, × 4.2. Balancers, rudimentary gills and limb buds may be present, or the balancer may be absorbed and the gills and anterior limbs well developed at this time. A and B are larvæ from the same egg mass, the larva shown in B having hatched January 5, 1930, a month later than the larva shown in A. C and D are larvæ also from a single egg mass, the second (D) having hatched six days after the first. Although the reduced balancers indicate that the last hatched larva is in a more mature stage of development, the larva is not as large as the one hatched nearly a week earlier.

sure complete saturation. The eggs, however, were not in contact with the water. In five of the seven dishes some of the eggs hatched, after remaining for some time on this saturated medium. Some of the larvæ made their way into the shallow water at the bottom of the dish. If they were unable to reach the water, as was usually the case, they seldom survived more than a few hours. We briefly summarize the experiments in this "b" series below.

Experiment 1b.

Forty eggs were placed on saturated sand on November 4. The first hatched forty-one days later, the last after fifty-four days, as indicated below.

December	14	17	24	25	26	27
Number hatched	1	2	1	3	1	1

Only 22.5 per cent of the eggs hatched. Some of the remainder lived in good condition until January 28 without hatching. One larva removed from the capsules on January 7 had lost the balancers and was developing the hind limb buds.

Experiment 2b.

Twenty-five eggs were placed on saturated sand on November 4. Only 28 per cent hatched, the first after fifty-two days, the last after one hundred days. Their hatching schedule was as follows:

December	25	28	29	Feb. 2	10	17
Number hatched	1	1	1	1	1	1

Two unhatched eggs were still alive on March 4. The last hatched, unlike the first hatched, had lost the balancers, differentiated four digits on the fore limb and had hind limb buds.

Experiment 3b.

Fifty-five eggs were placed on saturated sand on November 4. Only 10.9 per cent hatched, and these from thirty-three to fifty-four days after the beginning of the experiment.

January	6	12	18	19	23	27
Number hatched	1	1	1	1	1	1

All the larvæ which hatched had lost the balancers and acquired all four digits.

Experiment 4b.

Fifty-nine eggs were placed on saturated sand on December 4. Only 20 per cent hatched and these from twenty-one to thirty-six days later.

December–January	24	26	27	28	30	1	8
Number hatched	1	3	1	4	1	1	1

The first hatched had balancers, three digits on each fore limb, and lacked the rear limbs. The last hatched had lost the balancers, had acquired four digits on each fore limb and had developed hind limb buds.

Experiment 5b.

Forty-eight eggs were placed on saturated sand on December 4. Only 12.33 per cent of the eggs hatched and these from thirty-seven to sixty-nine days later. The hatching record follows:

January–February	9	21	23	27	29	10
Number hatched	1	1	1	1	1	1

One egg lived until March but failed to hatch. All the larvæ in this experiment had lost the balancers before hatching. The fore limbs had four digits.

Experiment 6b.

    Twenty-seven eggs were placed on saturated sand on December 4. None of these eggs hatched but one lived until January 12. One removed from the capsules on January 6 had lost the balancers and had differentiated all digits on the fore limb.

Experiment 7b.

    Thirty-seven eggs were placed on saturated sand on December 4. None of these hatched but apparently they all lived until February 13. One larva removed from the capsules on January 6 had lost the balancers and developed hind limb buds.

    Although all workers who previously had considered the eggs of this species have held that complete immersal in water was requisite for hatching, the results of this experiment show that this is not necessarily the case. Contact with a sufficiently damp medium was enough to hatch more than 13 per cent (13.39) of nearly 300 eggs exposed to it. This percentage would have been higher if we had selected only eggs swollen with moisture in their nests for the experiment (See series "a").

    The series "c" experiments demonstrate the ability of the eggs of *Ambystoma opacum* to withstand a great amount of desiccation. It will also be seen, from the results, that intra-capsular development was not always retarded by the dry medium on which the eggs were kept.

Experiment 1c.

    Nov. 4: Forty eggs placed on dry, packed sand.
    Jan. 4: Sixteen eggs still good. One removed from capsule had balancers and rear limb buds.
    Mar. 4: One egg left. On removal from capsule embryo was found still to have balancers, a rudimentary fourth finger and hind limb buds.

Experiment 2c.

    Nov. 4: Twenty-five eggs placed on dry packed sand.
    Jan. 7: All good. One removed from capsule had balancers and a rudiment of the fourth finger.
    Mar. 4: One egg left. On removal from capsule larva was found to have lost the balancers and to possess a developed fourth finger and buds of the hind limbs.

Experiment 3c.

    Nov. 4: Fifty-five eggs placed on dry, packed sand.
    Jan. 4: Eighteen left. One removed from capsule had traces of balancers, fourth finger and hind limbs rudimentary.
    Mar. 4: Two left. The larvæ removed had the barest rudiments of balancers. The fourth fingers were rudimentary and the hind limbs still in bud stage.

Experiment 4c.

    Dec. 4: Fifty-nine eggs placed on dry, packed sand.
    Jan. 6: Nine in good shape. One removed from capsule lacked the balancers; had a fourth finger and rear limb buds.
    Mar. 4: Desiccation had proceeded too far at this time.

Experiment 5c.
  Dec. 4: Forty-seven eggs placed on dry, packed sand.
  Jan. 6: Eleven in good condition. Balancers lacking on specimens taken from capsules at this point, four digits on fore limbs, rear limb buds appearing.
  Mar. 4: Four remaining eggs recently dead.

Experiment 6c.
  Dec. 4: Twenty-six eggs placed on dry, packed sand.
  Jan. 6: Sixteen appear viable. One removed from capsule lacked balancers and showed hind limb buds.
  Mar. 4: Four alive. Much desiccated. Balancers gone but fourth fingers and rear limbs still comparatively rudimentary.

Experiment 7c.
  Dec. 4: Thirty-four eggs placed on dry, packed sand.
  Jan. 6: Thirteen good. Example removed from capsule has no balancers but still had yolk sac; fourth finger rudimentary; no hind limb buds.
  Mar. 4: Two good. Traces of balancers in one, none in other. Both embryos showed rudimentary fourth fingers and hind limbs.

To summarize, of one hundred and twenty eggs over a month old at the beginning of the experiment, practically 40 per cent were capable of standing a month's rigorous desiccation. Slightly more than 3 per cent survived two more months. More than one-third of one hundred and sixty-six eggs at least two months old survived a month's desiccation. A little more than 3 per cent lasted another two months under the same conditions. We may assume from these results that the eggs are capable of withstanding the several periods of drought which take place in the field. Conditions there would hardly approximate the degree of aridity to which the eggs in this experiment were exposed. Under more normal conditions a much greater percentage of the eggs will last over similar periods of time.

The longest period on record for eggs remaining within the nest is fifty-two days, recorded by Brimley (1920). Bishop (1924) kept the eggs more than a month. Lantz (1930) reports one egg kept on land in the laboratory, in which the enclosed larva lived for two hundred and seven days. We have kept eggs of *opacum* in good condition for over a year in our ice box at temperatures of 7° to 10° C. Lantz (1930, p. 324) comments on his long-term eggs: "If, however, hatching is retarded too long, the larvæ weaken gradually and become incapable of normal development, even if they finally succeed in freeing themselves from their envelopes." In the case of eggs kept seven months in the ice-box at 7° to 9° C., a fair percentage hatched out and developed normally when placed in water, the larvæ being fed *Daphnia* and *Enchytræus*. Our eggs over a year old did not hatch, but the enclosed larvæ were alive.

In view of these findings we have endeavored to determine if *opacum* normally ever winters over in the field. In the Washington area the fall of 1930 and the winter of 1931, were unusually dry. Evidence that some of the eggs survived this drought was obtained during March 1931, when several batches

of viable eggs were found in the still dry breeding ponds. In the Dead Run Swamp area, on the Virginia shore of the Potomac opposite Plummer's Island, two batches containing seven and thirteen eggs were found on March 5. Two days later a batch of fourteen eggs was found in the same locality. All of these eggs contained living larvæ. Most of them hatched immediately upon being placed in water. Some of the eggs in each batch—one in the first, three in the second and three in the third—failed to hatch after remaining immersed in water for several days, although the larvæ were alive when removed from the capsules. Batches of twelve and fourteen eggs were found in Rock Creek Park breeding sites on March 12 and one batch of fourteen was found near Priest's Bridge, Md., March 14. Most of these were viable but, as with the other sets, a few in each batch failed to hatch after immersion, although containing living larvæ. Half of the eggs in the last batch were placed on wet blotting paper, the other half being submerged in 2 cm. of water. Except for the few which failed to hatch, both groups of eggs hatched within the same period of time, one hour. All of the larvæ from these wintering-over eggs were in an advanced stage of development, possessing well developed branchiæ and rear limb buds and having the digits of the fore limbs well developed. They were all in a much weakened condition and extremely thin. They readily responded to efforts to feed them with entomostracans but failed to thrive on a diet of enchytræids. The failure of some of the larvæ to escape the egg capsules is paralleled by our experience in the laboratory and shows that the encapsulated larva may reach such a condition, if hatching be delayed, that the hatching mechanism may become unable to function. The number of eggs in these delayed batches (average twelve) is much below that which we have shown to be average for normal clutches at or near the time of laying. Hatching due to chance wetting caused by rains and thawing, or destruction by enemies, or both factors, probably account for this reduction in number. Although no empty egg capsules were found with these eggs, decay over the long period of time or extreme desiccation may have accounted for their disappearance. Observations made during the fall of 1931 in the Washington area indicate that amphibian and insect enemies destroy a high percentage of the eggs. These observations are discussed above.

In the present paper we are not describing either the early development of the egg or the later larvæ secured from the ponds. Such descriptions to be of value should point out the resemblance and differences between these stages in the life cycle of *opacum* and similar stages in other species of *Ambystoma*. At the time of writing we do not have adequate comparative material at hand. Mr. J. A. Weber has secured metamorphosing *opacum* at Miller's Place, July 4, 1929, and we have many records for *opacum* larvæ from Long Island ponds during the spring months.

The purpose of this paper has been to report in detail our observations on some of the more distinctive features in the life history of *A. opacum*. The most unusual feature of this life history is the habit of laying on land eggs which are destined to produce aquatic larvæ. It may be of interest to inquire further into the probable significance of this habit in the economy of the species.

Dunn (1917) has suggested that this terrestrial stage in the life cycle may represent an adaptation to the Atlantic coastal plain, with its conditions of

flood and drought. It does not seem to us that the distribution of the species lends support to this view. The species occurs throughout most of the Piedmont plateau and is found in the New England upland and in various areas in the interior lowlands. Its requirement of a semipermanent pond, which must contain water during at least the winter and spring months, is better fulfilled by the irregular topography and extensive drainage systems of such a region as the Piedmont plateau. In the coastal plain, with its relatively much more porous top soil and its greater rate of evaporation, these ideal conditions are less likely to be found. One of us has recently collected *opacum* in some numbers at Biloxi, Mississippi. The species was found thirty miles from the coast in hardwood swamps. According to Allen (1932) this species does not occur at all on the sandy coastal plains skirting the gulf coast at Biloxi. Here *Ambystoma talpoideum* breeds in small pools. This species which flourishes in a sandy coastal plain has not given up the water breeding habits of *A. maculatum*.

Those who have had field experience with species of *Ambystoma* have often found that different species occurring in the same area breed either in different though perhaps adjacent ponds, or if in the same pond, at different periods. Smith (1911) pointed out that, in the vicinity of Ann Arbor, *A. maculatum* and *A. tigrinum* breed in different ponds, "each species occurring to the exclusion of the other." He noted only a single exception to this rule. We have found the rule to hold for the Syosset area of Long Island. Piersol (1929) shows that *A. jeffersonianum* breeds before *A. maculatum*, in the pools around Toronto. The larvæ of the *A. jeffersonianum* are well developed by the time those of *A. maculatum* hatch, and feed largely upon them. This condition is balanced by the factor of pathologic polyspermy in a large proportion of the eggs of *A. jeffersonianum* induced by the low temperature of the water in which they are laid. The irregular development which follows destroys many of the eggs of *A. jeffersonianum.* Since this species is known to be an earlier breeder than *A. maculatum* throughout the common range of the two species, the temperature factor would appear to keep the more voracious *A. jeffersonianum* in check. Throughout much of its range *A. opacum* occurs in the same area with *A. maculatum.* The latter species produces more eggs than the former. Wherever the forms occur together as near Coram, Long Island, and in the Washington stations, they may breed in the same ponds. It may well be that after the perils of the several preceding months, the well-developed larvæ of *A. opacum* are so greatly reduced in numbers that *A. maculatum* is able to continue successfully. Such factors as active enemies in the more southern portion of the range, extreme cold with resultant ice in the northern parts of the range, together with periods of too prolonged drought throughout the range, must reduce greatly the number of *opacum* larvæ which live to transform. Since both species are so exceedingly abundant in the same area one would look for some such balance, as the larval habits appear to be the same. The adults of the species range over a large area but the number of available breeding sites within the area is comparatively small and would result in much concentration were the spawning dates to occur simultaneously. Therefore, we look upon the fall breeding and terrestrial egg-laying habits of *opacum* as an adaptation to avoid competition with other species of *Ambystoma.*

Mechanism of Hatching

Previous to the publication of our preliminary note (Noble and Brady 1931) on the hatching mechanism in *A. opacum* it was generally assumed that the larvæ escaped from the egg capsules by mechanical means. Bishop (1924) states "the embryo breaks out" of the capsules. Brimley (1920) mentions the larvæ as "trying to hatch out" before the eggs were placed in water. Lantz (1930) assumes that the egg capsules are weakened by the water. He states:

"With regard to the mechanism of hatching itself, it seems probable that a large part is played by sudden swelling of the eggs through rapid absorption of water up to the bursting point of the membranes. Eggs immersed in water in the earliest stages will develop normally, but the larvæ seem incapable of hatching or they do so very late, when obvious disintegration of the membranes has taken place."

Since larvæ may hatch either head or tail first it appeared highly probable that the capsules had given way at their weakest point to the pressure exerted by the half-coiled embryo within. However, in the experiments reported above we found that in many clutches of eggs immersed in water some of the eggs will fail to hatch even though they contain living embryos. It seemed strange that in eggs developing side by side all should not hatch at approximately the same time if the only factor concerned was that of water absorption with an accompanying disintegrating effect upon the capsules. Further, as stated above, we observed many instances of eggs hatching on damp sand. These observations forced us to reject the conclusion reached by Lantz that:

"The conditions required for normal hatching seem to be for the eggs to remain on land in moderately humid surroundings until mature, and then to be suddenly immersed in water."

The eggs obviously could hatch under other conditions, and moreover many eggs did not hatch when placed in the conditions which Lantz indicated. This caused us to search for another mechanism of hatching. One of us had previously shown that in *Alytes*, the Midwife Toad, the eggs could also be induced to hatch on land. In this species the larvæ were freed from the egg capsules apparently by the digestive action of a series of unicellular glands scattered over the snout (Noble 1926). These hatching glands had been previously described in an aquatic frog, but were unknown in any urodele until Wintrebert (1928) reported them in *Ambystoma mexicanum*. Sections of the larval *A. opacum* just before hatching reveal large numbers of these unicellular glands present over the snout and extend along the sides and top of the head. None are present on the tail.

The hatching glands are large epidermal cells which stain intensely with plasma dyes (Fig. 93). In Mallory's anilin blue connective tissue stain, following fixation in Zenker's solution, the hatching glands stain yellow while the remainder of the epidermis takes a bluish stain. The hatching glands are not to be confused with the only other large cells in the epidermis, namely the Leydig cells. In haematoxylin-eosin preparations the latter stain feebly and show a reticular cytoplasm while the hatching glands stain intensely and have a finely granular cytoplasm. The hatching gland cells are widely scattered over the

head in *A. opacum* and only rarely do two of them lie side by side. In *A. opacum* each cell has its base in the germinative or basal layer of the epidermis while its distal end reaches the surface. The secretion within the cell crowds the nucleus proximally. A cuticle is absent in the hatching gland cells. In larvæ fixed at the moment of hatching some of the glands may be seen discharging
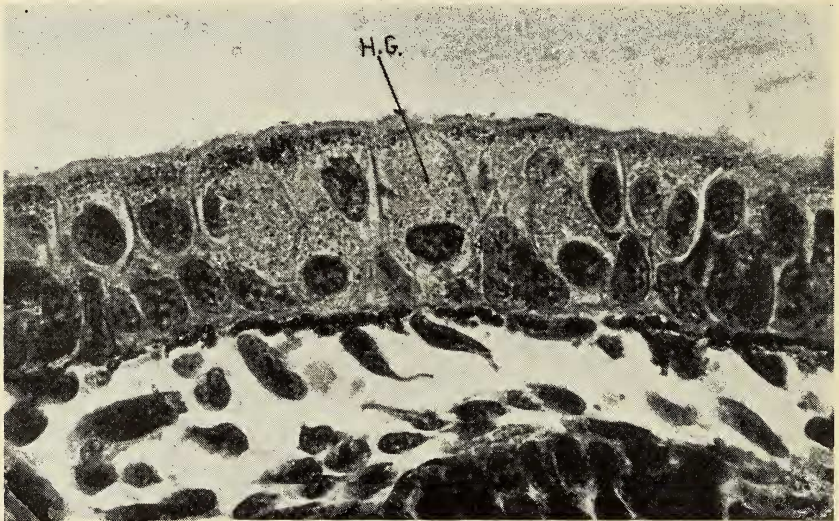

Fig. 93. Vertical section of the integument of the head of a larval *Ambystoma opacum* immediately before hatching, × 500. The unicellular hatching glands (H. G.) reach the surface and lack the cuticle which forms a conspicuous margin to the superficial epidermal cells. The function of the hatching glands is to digest an opening through the egg capsules which will permit the escape of the larva.

their secretion on the surface. In larvæ treated with pilocarpine the majority of the hatching gland cells have collapsed. A few days after hatching these gland cells have entirely disappeared.

We have studied sections of the heads of several other species of Caudata at the time of hatching. We have found unicellular glands similar in structure to those of *A. opacum* in *Hemidactylium scutatum, Plethodon cinereus, Desmognathus fuscus* and *Amphiuma means,* species which hatch out on land, and also in *Ambystoma maculatum* and *Necturus maculosus* which hatch in the water. Hatching glands are known not to occur in frogs which hatch fully formed from the egg capsules aided by a cornified egg tooth. It is highly probable that they occur in all other Amphibia.

That these unicellular glands really function as hatching organs has been shown by Wintrebert (1928) in the case of axolotl. In view of the fact that the environmental conditions are so different in the case of *A. opacum* we have endeavored to determine by experiment their function in this form. In one series of experiments we cut the larvæ free from their capsules before hatching.

After anæsthetizing them in a 1 to 4000 solution of chloretone we dipped them for a few minutes in a 1 per cent solution of pilocarpine hydrochloride. A series of eggs was then placed in watch glasses on slightly dampened Scottissue and each brought in contact with the head of one of the larvæ. Within a few minutes all the eggs thus manipulated had hatched, but none of the controls which had not been brought in contact with anæsthetized larvæ.

In order to exclude the possibility that the larvæ inside of the capsules had influenced their own hatching we performed a second series of experiments. The larvæ were removed from the capsules before hatching and their heads and tails cut off. When the tails or bodies were immersed in a 1 per cent solution of pilocarpine and brought in contact with the empty egg capsules lying on damp Scottissue no modification of the capsules was noted even after three hours of contact. When the heads were similarly treated and their dorsal surfaces brought in contact with the capsules a hole was digested either completely or partly through the capsules in the same period of time. The result was the same whether the treated head was brought in contact with the inner or the outer capsule. When the isolated heads were merely immersed in water and then brought in contact with the egg capsule no disintegration occurred during the three hour period. No change occurred in capsules immersed in water or in 1 per cent pilocarpine, alone, during this period.

We have studied the hatching of *A. opacum* both in water and in dilute solutions of pilocarpine. The embryo, or larva, at this time moves about violently within the capsules pressing the top of its head against the enclosing walls at many points. If the top of the head remains in one position for a short time the capsules will begin to soften and the outer surface becomes distended at this point. Fine white fibers appear in the capsules in this region, these being most numerous directly opposite the point of contact. If the embryo should move its position the area remains distended and probing with a needle reveals that this portion of the capsules has softened and lost most of its elasticity. If the tail should be thrust in one of these pockets it may break through to the outside before the head, which has begun its digestive action on another part of the capsule, has emerged at the new point. We have observed cases where tail and head hatched out at the same moment and the larva remained for several minutes threaded through the capsules. In a few cases the embryo moved so frequently that a large part of the inner capsule became opaque before hatching. In all these cases, however, it seems clear from the experiments reported above that the head alone initiates the hatching process.

Since the enclosed embryo is usually very active at the time of hatching, it must exert a certain amount of pressure upon the egg capsules. Eggs brought in contact with filter paper moistened with 1 per cent pilocarpine hatch in a shorter time than is required for egg capsules to become perforated, when they are moistened with the same solution and brought in contact with an isolated head. This apparently is due to the fact that the embryo is exerting more pressure against the capsules than the isolated head is able to do. We have placed a large series of eggs on Scottissue moistened with 1 per cent pilocarpine. One set of controls was placed on Scottissue moistened with a similar amount of salt solution. Another set was placed on Scottissue moistened with the same

amount of water. Only the first set hatched. Many of these began to hatch within a few minutes and all the larvae had escaped within two hours. An isolated head we had previously found would not invariably digest its way through the capsules within two hours after being placed upon them. Some digestive action would have been begun during this period but often it would not be completed.

We have attempted to arrest the hatching process by treating hatching eggs with dilute solutions of atropine sulphate. Embryos which had begun to digest through the capsules after a brief immersion in a 1 per cent solution of pilocarpine were immersed in a 1 per cent solution of atropine. Hatching continued without a marked delay. When eggs were placed on Scottissue moistened with atropine solutions of 5 per cent to 1 per cent no hatching occurred over a period of several hours.

The egg capsules of any species of Amphibian are very rarely found in nature after the escape of the larvæ. It has been shown above that hatching is accomplished by the digestive action of the integument covering the dorsal surface of the head of the embryo. The only structure found in this tissue and not in the integument of the tail and body are the unicellular glands described above. Therefore, we have concluded that they digest the egg capsules at the time of hatching. It might be assumed that the digestive action is continued after hatching and this leads to the disappearance of the egg capsules of *A. opacum* and other Amphibia. We have tested this hypothesis by placing a series of seven recently hatched larvæ in a watch glass with their egg capsules and a solution of 1 per cent pilocarpine. After a week the egg capsules showed no marked disintegration. A second series of egg capsules from which larvæ had hatched was placed in water and a third series of similar egg capsules in a 1 per cent solution of pilocarpine. This experiment was begun on December 13 and on the following January 29 very little disintegration had occurred. The inner capsules were removed from the outer and found to be still intact. These experiments show that the influence of the hatching gland is extremely local. They also show that in contrast to the views of Lantz, water *per se* has little disintegrating effect upon the egg capsules. It would appear that other agencies, possibly bacteria, cause the rapid disappearance of the egg capsules in nature.

CONCLUSIONS

1.—*Ambystoma opacum* lays its eggs under leaf mold, sphagnum, dry water weed or other cover in situations which will be flooded by the winter rains.

2.—Egg-laying may occur as early as September 18 on Long Island or as late as October 4 in Westchester County, New York.

3.—The female may dig a shallow depression for a nest or may utilize crayfish burrows or other natural cavities.

4.—The egg-laying site varies from year to year. The degree of moisture in the soil has an influence on the selection of a nesting site.

5.—The breeding female is not at home in the water and will drown if confined in this medium.

6.—There is considerable variation in color in the adults. The breeding males are whiter above than the females but there is no sexual difference in color pattern.

7.—The adults migrate to suitable breeding sites about the middle of September. The males become sexually active earlier than the females and excite a group to sexual activity by engaging in a series of rubbing movements which are exactly the same whether directed toward male or female.

8.—It is probable that the odor of the female excites the male to the production of a spermatophore.

9.—The behavior of the male induces the female to follow him and this brings the female into the right position for picking up the spermatophore. The female, however, may find and cover any spermatophore after she has been excited.

10.—The spermatophore of *A. opacum* has a distinctive form, its head being roughly quadrangular. The spermatozoa are frequently directed outward. They are held together by pelvic gland secretion. The stalk of the spermatophore is formed by the mucous secretion of the cloacal glands and a supporting framework of eosinophilic secretion produced by glands lying on either side of the pelvic gland.

11.—The spermatozoön agrees essentially with that of *A. mexicanum* except that it has a shorter lash to the tail, a longer head, and no barb.

12.—The female lays from 75 to 232 eggs, with 150 for an average. The egg has four capsules. It is capable of great desiccation without destruction. Eggs found in normal nests in nature will absorb approximately their own weight of water within twenty-four hours, when immersed in that medium.

13.—After laying, the female usually remains with the eggs for a period of at least several weeks. She may or may not return to them once she has left them. She does not forage for food during the early part of the brooding period. Eggs brought into the laboratory do no appear to be attractive to the female.

14.—In the field the female deserts the eggs after approximately a month of brooding. Unguarded eggs may be eaten by salamanders, insects, or other enemies.

15.—Eggs will hatch on land as well as in water. Moisture facilitates development. Desiccated eggs do not hatch as quickly as swollen eggs when im-

mersed in water, and the larvæ that escape are more embryonic than those from swollen eggs.

16.—Unhatched larvæ may winter over on land if the winter is unusually dry. Eggs containing such larvæ were collected in the field March 14. Others kept on ice over a year contained living larvæ.

17.—The terrestrial stage in the life cycle of *A. opacum* is an adaptation permitting the species to compete successfully in the same region with other species of *Ambystoma*.

18.—Hatching is accomplished by the digestive action of a series of unicellular glands scattered over the head of the embryo. They occur in other species of salamanders which lay eggs on land as well as in species laying them in water.

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